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DECREASED INCIDENCE OF LIMBIC MOTOR SEIZURES FOLLOWING TWENTY PAIRINGS OF SUBCLINICAL LITHIUM-PILOCARPINE INJECTIONS AND A COMPLEX "BURST-FIRING" MAGNETIC FIELD

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ABSTRACT

An experiment was designed to determine whether temporal contiguity between subclinical dosages of (sub. cu.) lithium/pilocarpine and exogenous magnetic fields would alter the probability for overt limbic motor seizures. Magnetic fields that simulated (computer generated) the temporal structure of burst-firing amygdaloid neurons were presented for 1 s every 4 s for 4 min per session. After 20 pairings (40 days), injection of 3 mEq/kg of lithium and 30 mg/kg of pilocarpine evoked the normal limbic seizures (rearing and forelimb clonus) in 100% of the controls but in only 68% of the rats that had received the magnetic fields.

INTRODUCTION

The Goddard effect (1) is a robust demonstration that intermittent, subclinical neuroelectrical stimulation within critical brain structures can evoke a progression of transencephalic changes. Approximately 0.5 min of electrical stimulation per day, which constitutes less than 0.05% of behavioral time, is sufficient to initiate and to maintain the kindling process (2,3). Chemical kindling, such as repeated central injections of muscarinic (cholinergic) compounds, has also been documented (4).

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The possibility that synergistic interaction between mundane pharmacological treatments and very weak current induction by complex, environmental, extremely low-frequency (ELF) magnetic fields has recently received public attention. In response to that concern, we designed an experiment that would determine whether brief, but repeated exposures to complex magnetic fields could conspicuously lower the thresholds for limbic seizures. A comparable change in the electrically sensitive human brain could explain the frequent phenomenological (complex partial epileptic-like) experiences that are popularly associated with proximity to ELF magnetic fields.

The systemic (sub. cu.) injection of lithium (3 mEq/kg) followed 4 h later by 30 mg/kg of pilocarpine evokes consistent rearing and forelimb clonus within 20–40 min (5); the effect is indiscriminable from a single I.P. injection of 400 mg/kg of pilocarpine. Only rats that display this behavior, which is most similar to stage IV kindling (3), show a specific pattern of necrosis within diencephalic and telencephalic structures (6). As anticipated by McIntyre and Plant (2), the temporal evolution of the multifocal brain injury could be initiated by muscarinic processes (or inhibited by a norepinephrine effect) within the amygdaloid-piriform cortex.

We decided to test, by inference, the specific hypothesis that repeated, sub(overt) seizure, central cholinergic activation could be "kindled" by temporally contiguous applications of a weak (50 mG; 5 μT) exogenous magnetic field with a muscarinic agent. Because the potential for resonance (7,8) between the applied field and endogenous neuroelectrical properties promotes interaction and hence reduces the field strengths required to evoke an effect, a pattern was selected that simulated limbic burst-firing. This particular ELF field, when presented for 1 s once every 4 s for 20–30 min, was shown (9) to elevate nociceptive thresholds in rats (at least over three successive daily trials) and to alter the affective dimensions (10) of spontaneous narratives for human subjects. If the hypothesis by Ossenkopp and Cain (11,12) is generalizable, then stimuli that elevate endogenous opiates should increase the electrical lability of the brain and hence its susceptibility to seizures.

MATERIALS AND METHODS

In two separate experiments, a total of 24 male Wistar albino rats (obtained from Charles River Laboratories, Quebec) served as subjects. They had been acclimatized to 20°C and housed (three per cage) in standard wire cages for at least 4 weeks. The light:dark cycle was 12:12 h with lights on from 07:30 to 19:30, local time. Rat chow and water were available *ad libitum*. At the beginning of the experiment, the mean and SD for the rats' body weights were 505 g and 83 g, respectively (110–140 days old).

Once every second day, for a total of 20 sessions, each rat was injected sub. cu. with 1.5 mEq/kg of lithium chloride (1.5 mEq/cc). Four hours later it was injected sub. cu. with 15 mg/kg of pilocarpine (15 mg/cc) and then exposed to either the magnetic field or control condition for a total of 4 min (2 × 2-min exposures; the second exposure was given after all rats in a given experiment had completed their first experimental or control exposure, i.e., about 30 min). Each rat always received the same treatment. Half the number of rats in each experiment received the magnetic field treatment while the other rats served as controls.

For the magnetic field treatment, the rat was exposed singly within a 17 cm by 30 cm by 13 cm (deep) plastic cage that was placed between two solenoids. An IBM-compatible personal computer was used to generate the burst-firing wave pattern (95% fidelity as determined by external solenoid induction within the exposure area), through Hercules complex software that was connected to the solenoids through a differential (d.c. coupled) amplifier. Because there were 250 successive temporal points in the waveform and we selected 4 ms as the duration of each point, the burst-firing pulse duration was 1 s. The pulse was delivered for 1 s once every 4 s; there were 20 peaks per second (see Ref. 9 for a diagram of the wave structure). A Metex N3800 meter coupled to a magnetic sensor indicated a field strength of 50 mG (5 μT). Control rats were placed in the apparatus; although the computer was operative, it was not activated (no field). At the end of the exposure period, the rat was returned to its home cage.

On the day of testing, each rat was injected sub. cu. with 3 mEq/kg of lithium and then 4 h later with 30 mg/kg of pilocarpine. They were then placed in our standard observation cages (13). The seizure onset time (SOT), which was defined by rearing, forepaw clonus, and falling backward, was recorded to the nearest second. If the rat had not displayed any overt signs of this activity within 70 min (e.g., >4 SD of the mean), it was designated as nonseizured. Because the results of both experiments were similar, the data were pooled. All analyses involved SPSS software on a Vax 4000 computer.

RESULTS

Whereas all of the rats that had been exposed to the control condition exhibited seizures, only 8 of the 12 experimental rats displayed an overt seizure. Chi-squared analyses indicated that this disproportionate incidence was statistically significant (chi square = 4.80, $p < .05$; phi = 0.44). If the rats that did not display overt seizures were given the default SOT, i.e., 70 min (end of observation period), the experimental group exhibited significantly [$F_{1,22} = 8.06$, $p < .01$] slower SOT [45.7 (18.2) min relative to the controls 29.4 (7.8) min]. There was no statistically significant difference [$F_{1,22} = 1.19$, $p > .05$) between the experimental and control groups with respect to body weight [524 (81) and 487 (86), respectively].

SOTs for only the rats that exhibited overt forelimb myoclonus, rearing, and falling were also compared. Although the SOTs (means and SDs in parentheses) were delayed in the group that received the magnetic field treatment [33.5 (3.7) min] compared to the control group [29.4 (7.8) min], one-way analysis of variance [$F_{1,18} = 1.87$, $p > .05$] demonstrated that the difference was not statistically significant; however, the magnetic-field-exposed rats exhibited significantly less variability in SOT (Cochran's $C = 0.81$, $p < .05$) than the control group.

DISCUSSION

The results of this study did not support the hypothesis that temporary contiguity between electrical currents induced by this particular weak magnetic field and

subclinical muscarinic stimulation facilitates kindling. Instead, the group that was exposed to the burst-firing pattern magnetic field for 1 s every 4 s for 4 min after the injections of 15 mg/kg of pilocarpine exhibited elevated seizure thresholds. Failure to display overt forelimb clonus and rearing within 70 min after injection of 3 mEq/kg lithium and 30 mg/kg of pilocarpine for this sex and age range is extraordinarily rare (99.5% occurrence is typical). That the rats that had been exposed to the magnetic field treatment did not seizure after the observation period was indicated by their normal food and water consumption during the subsequent days. If delayed limbic seizures had occurred, it would have been invariably reflected in subsequent hypophagia and adipsia (5).

The inhibitory effect of this particular magnetic field structure on the precipitation of limbic seizures is vectorially similar to other studies. Ossenkopp and his colleagues have shown that a 1-h exposure to 60 Hz, 1.85 G (185 μT) magnetic fields inhibited the onset of pentylenetetrazol-induced seizures (12). Even weaker intensity (0.01 G; 1 μT), 100-Hz magnetic fields prior to induction can elevate thresholds in rats that are susceptible to audiogenic seizures (14). The pulse pattern appears to be important since application of 40-MHz radiofrequency fields between 10 and 20 W/m and (pulse) modulated fields between 2 and 30 Hz (15) primarily inhibited electrical seizures that were directly measured from the amygdalohippocampal region.

The neurochemical or micromorphological process that mediated the inhibitory effect in the present study appears to have been an all-or-none phenomenon since rats exposed to the field either did not seizure or exhibited SOTs that were not significantly different from those of controls. If the process is similar to the effects of, for example, clozapine (16), the magnetic field treatment may have induced a compensatory increase in the incidence of earlier, less conspicuous stages of epileptiform activity. In the human analog, this would be reflected as a decrease in the frequency of overt paroxysmal motor patterns but an increase in the frequency of cognitive and perceptual anomalies.

Inhibition of the duration of afterdischarges during routine amygdaloid kindling was also reported by Ossenkopp and Cain (11) when they exposed rats to 60-Hz, 1-G (100 μT) magnetic fields for 1 h before daily brain stimulations. They hypothesized that the effect was due to the inhibition of endogenous opiates (17) by the applied ELF magnetic fields. In the present study, a magnetic field pattern that has been shown to elevate nociceptive thresholds (9), which is presumably associated with increased endogenous opiate activity, also retarded seizure incidence. Because no direct measurements of these neuropeptides were taken, their involvement is, at present, only speculative.

Although our results appear contradictory to the initial concept that was developed by Ossenkopp and his colleagues (18), the duration of field exposure could be a mediating factor. Elevated nociceptive threshold with the burst-firing, pulsed field occurred after 20 min of exposure over 3 successive days (the limit of the study) in the experiments of Fleming *et al.* (9) whereas, in the present study, the rats were exposed for only two 2-min periods (separated by about 30 min). If there is a nonlinear relationship between exposure duration and the ostensible opioid effect, then this contradiction might be resolved. The duration of this study (40 days) was well within the range of time required to synthesize receptors (*de nouveau*) or to alter receptor densities. If receptor processes were involved, then repeated exposures to this effective magnetic field may have been associated with tolerance.

Despite the consistent demonstration that ELF magnetic fields can influence seizure probability, a conspicuous discrepancy still exists. Whereas increased daily geomagnetic activity is often associated with responses that are typical of lowered seizure thresholds (13,19), the application of artificial magnetic fields has been more frequently associated with behaviors that suggest elevated seizure thresholds. Both our published and unpublished data indicate that the probability of seizures (stereotyped head nods, forelimb clonus, "absences," and automatisms) increases in chronically limbic epileptic rats when the daily geomagnetic activity exceeds 20–25 nT particularly if the global activity during the previous 3–5 days has been below that value. Most experimental studies employ field strengths that are at least 100 or more times greater than natural ELF fields. We have also noted one other operational difference. For correlational studies the association between increased seizure probability during the local day is most frequently associated with the ambient geomagnetic activity of the previous nocturnal period.

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